U.S. PATENT APPLICATION

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Invention: METHOD AND DEVICE FOR THE DETECTION OF MICROORGANISMS ·
BY FIBER OPTICS

SPECIFICATION

METHOD AND DEVICE FOR THE DETECTION OF MICROORGANISMS BY
FIBER OPTICS

The present invention refers to a method and device
for the detection of microorganisms using a combination of
microbiological procedures with devices constructed with
fiber optics and related components.

The microbiological procedures enable the
microorganisms to be selectively cultivated and these,
when in physical contact with a conveniently constructed
fiber optic circuit, permit the detection and monitoring
of the microorganisms in a fast and accurate way. The
device overall is physically consisted of three
subsystems:

BACKGROUND OF INVENTION

Microorganisms are life forms that despite their
micrometrical size scale significantly affect human life.
They may constitute viruses, bacteria, fungi, protozoa and
algae. They are present in solids, liquids and air; and in
all cases it may be of vital interest or importance to
detect the specific presence and/or to monitor,
qualitatively or quantitatively, the growth over time of
one or more of these microorganisms. Many of them are of
use to various types of industries, such as food
industries, brewery, viticulture, pharmaceutical, etc.,
for the catalysis of many biochemical reactions of
commercial interest, amongst others. Other microorganisms
do not seem to serve any other practical purpose, but are,
in principle, harmless to human health. However, some of

these microorganisms may become dangerous, for a series of reasons, after undergoing certain types of biological transmutations and whereupon they are termed pathogenic. These pathogens may exist and develop in various

5   environments. Other types of microorganisms may be intrinsically dangerous, meaning that they are naturally constituted as noxious to human health. Overall, the presence and the concentration of some of the referred microorganisms directly affect: (i) the quality

10   (potability and suitability for bathing)of the water for consumption in major urban centers and seaside resorts, possibly causing a series of diseases, such as diarrhea; (ii) the quality of food, which may generically cause food poisoning, and, particularly, "hamburger disease" and

15   (iii) the quality of air in clinics, outpatients and hospitals where the presence of aerobiological microorganisms constitutes, for example, an important vector for hospital infections. In any of these cases, it may be of great interest or vital importance to detect the

20   presence of certain microorganisms in real time or almost immediately, and consequently to monitor their evolution over time aiming to infer some useful information. At present, the conventional techniques for surveying microorganisms are closely linked to laboratory and

25   outpatient procedures and based in selective biological culture and in the use of microscopy for direct visual observation. These techniques are reasonably complex, as they demand from the operator significant intervention and skill in order to obtain dependable results, and typically

30   require 1 to 10 days for completion. This means that the conventional techniques require an average of 72h between

the capture of the microorganism, its isolation, identification and consecutive monitoring of its evolution over time. The standard procedures concerning bacteriological tests are furnished by the American Public

5   health Association (APHA). All these procedures require incubation in a culture medium to produce an adequate supply of microorganisms for various analyses at the end of the test. Apart from which, the conventional techniques use equipment that is not particularly low cost, making

10 its employment difficult or unviable for widespread surveying, in other words, for detection/monitoring of microorganisms in more than one place simultaneously.

There are other more modern procedures concerning biological detection, such as: radiometry,

15 electrochemicals, chromatography, chemiluminescence, pulse field electrophoresis and fluorescence. Concerning the techniques mentioned, there are some practical restrictions such as, for example, their success is highly dependent on the quantity of bacteria that can be

20 concentrated in the test sample. This procedure, generally, requires a minimum of 10^4 bacteria. In molecular reactions, the test procedures are particularly liable to cross contamination by other molecules or molecular fractions.

25 In addition, optical preventive techniques can be employed, aiming the elimination of pathogenic microorganisms, highlighting, for example, the use of ultraviolet light as a germicide, avoiding the infection of a determined environment or a material medium. On the

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other hand, the medical knowledge for fighting pathogenic microorganisms that had already infected the human body, especially those originating from hospital infection, food poisoning and water contamination, is vast but incomplete. Such microorganisms may also become resistant to any known drug due to their possible biological mutations. Recently, some success has been achieved with patients infected with these microorganisms using photochemical drugs activated by electroluminescent semiconductor diodes of high optical power with the correct wave length, permitting the total elimination of these microorganisms, without, in principle, harming the patient. This is the case, for instance, of the procedures recently developed and described by Pearce *et al* (H Pearce, M. Messager and J.Y. Maillard. "Effect of biocides commonly used in the hospital environment on the transfer of antibiotic-resistance genes in *Staphylococcus aureus*". J.Hosp. Infect.43.2, pp.101-107, 1999).

The optical surveying of microorganisms, which is the object of the invention herein, finds its place between the preventive techniques for the reduction/elimination of microorganisms and the techniques that aim to eliminate pathogens that have already infected the patients.

The essential characteristics of any biological sensor are its selectivity, sensitivity, resolution and response time, characterized by reactive recognition based on the type of test and the choice of detection technique. At a second level, there must be a biological surveying technology available that is robust, practical and of low

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cost, so that it may be employed in the field. Presently, various techniques and correlated devices are available, many of these are still at laboratory level whilst others are commercial, all aiming the detection and monitoring of microorganisms.

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Microscopy, as already mentioned, constitutes a fundamental analysis tool in microbiology, not only for the detection/monitoring of microorganisms, but also for the basic study of these. In the same way, there is a method of surveying bacteria involved in processes of hospital infections presently in use, known as pulsed field electrophoresis in, which is capable of tracking with precision the microorganisms involved, mapping and evaluating the level of environmental impact. However, it presents the disadvantage of the analysis taking approximately 7 to 14 days, as described by Birron and Lai (B. Birron and E. Lai. "Pulsed field electrophoresis: a practical guide". Academic Press, San Diego, 1993).

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With the aim of undertaking microbiological detecting and monitoring in an automatic and selective manner, the concept of the biosensor arises. A biosensor may be understood as a discreet electrical and/or optical component built based on the integration of biological materials with inorganic materials. A biosensor, in practice, is therefore capable of producing an analog electrical or optical signal when placed in contact with some specific subject, whether by its presence or any chemical-biological alterations occurring from it. A biosensor may be considered as a biologically active

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element, however, it requires connection, in some form, to a larger configuration, so that the electrical or optical analog can be adequately demodulated. The set-up thus formed is called a biological sensor. Fiber optics, in a general manner, may be used in the construction of biosensors, which means that through some appropriate technique, biological materials should be integrated to the sheath or core of the fiber (generally of glass or plastic) composing, therefore, an optical biosensor. In this manner, the light propagating through the core of the fiber can interact with the biological material (an antibody, for instance) integrated through a coupling by evanescent-field. The evanescent wave biosensor can induce modulation of intensity, complete phase, wave length or polarization of the propagating optical signal, or a fluorescent signal can be generated in function of a photo-biological reaction where part of the energy of this signal propagates itself through the optical fiber. Evanescent-field biosensors are described in the state of the art (J. S. Schultz. "Biosensors". Scient. Amer. pp.64-69, 1991.; J.P. Golden, G.P. Anderson, R.A. Ogert, K.A. Breslin and F.S. Ligler. "An evanescent wave fiber optic biosensor: Challenges for real world Sensing". SPIE. 1796. 1992. pp. 1-8; S.P.J. Higson and P.M. Vadgama. "Development o an evanescent wave fiber optic biosensor. Med.& Biol. Eng.& Comp. 32.1994. pp. 601-609).

Biological sensors based on evanescent wave are, in a general manner, dependent on a combination of various technologies of biological, physical and chemical natures. In this sense, the North-American patents US4,447,546 and

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US4,558,014 describe the techniques of spectroscopy by total reflection (TRS), where the use of evanescent wave to excite a fluorescently linked analytic and, consequently, detect the resultant fluorescence is

5   claimed. Other patents describe methods to improve measurement precision. This is the case of patent US5,631,170. The method involves marking the biosensor with fluorescent antibodies, in other words, antibodies linked to dye molecules. Provided that the antibodies

10   mentioned are not linked to the antigens (subject), the dye molecules will not be excited by the evanescent-field. However, when the biosensor is in the presence of the specific antigen to be detected, the propagating light will be capable of inducing fluorescence in the dye

15   molecules. Another method is that which is described in patent US4,242,447, that bases itself on the detection and quantification of bacteria in a liquid sample characterized by fact that this sample contains an agent capable of inducing the production of an enzyme in the

20   bacteria. This enzyme shall be capable of reacting with a fluorescent conjugate ingested by the bacteria, in such a manner as to release its fluorescent part, and this total fluorescence is measured and compared to the total of bacteria present in the medium.

25   However, as previously mentioned, there is an urgent need for the development of a method for detecting microorganisms that is sensitive and has a quick response, without the inconvenients already mentioned, such as: necessity of a minimum of 10^4 bacteria, cross

30   contamination by other molecules or molecular fractions

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and minimum average time of 72h between capture of the microorganism and the monitoring of its evolution over time.

Thus, aiming to achieve this objective, a strategy of combining biological procedures with a fiber optic sensor with an evanescent-field was used, capable of detecting the microorganisms 4 to 5 times faster than when employing conventional procedures.

SUMMARY OF INVENTION

The aim of the present invention is the detection/monitoring of microorganisms found in air, water or foodstuffs, through the use of a fiber optic biosensor with an evanescent-field.

A first concretization of the present invention concerns a method for the detection of specific microorganisms through the application of an evanescent-field of a sensitive optical fiber, characterized by the stages:

(a) exposing the evanescent-field of the sensitive fiber optic using an appropriate technique based on physical and chemical properties;

(b) permitting immediate contact of the exposed evanescent-field obtained in the stage (a) with the sample to be examined, with the aforementioned sample having a form adequate so as to obtain the generation of an optical signal in response to the presence of microorganisms in the sample;

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(c) demodulating the optical signal generated in stage (b) and using this value to quantify the microorganisms through an appropriate method.

In a second concretization, the invention is directed to a composition for use in the detection of microorganisms characterized by incorporating a selective culture medium for microorganisms needing to be detected and reactants capable of altering the properties of the medium in a manner as to favor the interaction of the system fiber-microorganism.

In a third concretization the invention refers to a device for surveying microorganisms through the insertion of a sensitive fiber optic (11), with an adequately exposed evanescent-field, into a surface or volume of a biological culture medium (12) specific for the microorganism to be detected, comprising a demodulation system based on a fiber optic circuit and related components.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Shows the preferential assembly of the device for the detection of microorganisms through fiber optics employed in the present invention.

FIGURA 2: Shows the exit signals of the optical sensor with *Staphylococcus aureus* resistant to methicillin. Lines 1, 2 and 3 represent the exit signal and line 4 concerns the estimated number of bacteria.

FIGURE 3: Shows the exit signals of the optical sensor with *S. pneumoniae*. Lines 1, 2 and 3 represent the exit signal and line 4 concerns the estimated number of bacteria.

FIGURE 4: Presents data for the temporal optical response of the biosensor with *E. coli* 0157:H7.

FIGURE 5: Shows the time of remaining in the Lag phase *versus* the initial number of bacteria *E. coli* 0157:H7.

FIGURE 6: Shows data for the sensitivity of the biosensor during the Lag phase.

FIGURE 7: Shows a photograph obtained by electronic microscopy of *E. coli* 0157:H7 (Lag phase) in physical contact with the optical fiber.

FIGURE 8: Shows a photograph obtained by optical microscopy by phase contrast of *E. coli* 0157:H7.

DETAILED DESCRIPTION OF THE INVENTION

With the purpose of resolving the existing inconveniencies in the state of the art the present invention was developed, that consists in a sensor for microorganisms based on a technology composed of microbiological procedures combined with a fiber optic device.

The microbiological procedures consist in permitting the growth of a selective culture of a determined microorganism in an adequate support such as, for example, o Petri dish or a slide where the medium is composed of

nutrients appropriate to the growth and viability of the microorganism and their quantity, pH and temperature are precisely controlled. The choice of the selective culture medium will depend on the microorganisms to be monitored being, however, of common knowledge to experts in the field. The optimization of the culture medium shall be undertaken with specific nutrients, such as: substance(s) source(s) of nitrogen, for instance industrial residues rich in protein, soya protein, urea, yeast extract; (ii) substance(s) source(s) of carbon, for instance manitol, dextrose, sacharose; and substance(s) source(s) of micronutrients, selected from, for instance, mixtures of salts involving $MgSO_4$, $MnSO_4$, $ZnSO_4$, $FeSO_4$ and $CaCl_2$. As an option, it is possible to use reactants that are capable of altering the properties of the culture medium in a manner as to permit that the index of refraction of the subject is better detected. In other words, these reactants will favor the fiber-microorganism interaction. In this manner, it is possible to have a composition containing selective culture medium for the microorganism chosen for detection and reactants capable of altering the culture medium so as to permit that the index of refraction of the subject is better detected.

On the surface or inserted in a volume of the aforementioned biological culture medium is a segment of optical fiber termed sensitive fiber optic that is then in direct physical contact with the subject.

The basic geometric structure of an optical fiber consists of a concentrically cylindrical core and sheath.

Light is propagated through the core, being almost
entirely spatially combined there, which characterizes
one or more means of transversal optical propagation.
Basically, what ensures the confinement and the
5   propagation of light in an optical fiber is the fact that
its core possesses an index of refraction that is superior
to the sheath, as it permits the occurrence of the total
internal reflection phenomena. An optical fiber may
support the propagation of only one mode (monomode fiber)
10   or two or more modes (multimode fiber). In either of the
cases a part of the propagating luminous energy always
extends beyond the sheath decreasing in amplitude in an
exponential manner along the radial co-ordinates. This
part of light that extends beyond the sheath is termed
15   evanescent-field. The sheath of an optical fiber may
already be sufficiently thin, or have its thickness
decreased in such a manner, that the evanescent-field
becomes exposed externally, in other words, in a manner
that permits that the light suffers the phenomena of optic
20   tunneling through the sheath and can interact with the
outside environment.

Alternatively, the sheath of the optic fiber may be
completely removed so as to directly expose its core. In
this last case, the external medium alone assumes the role
25   of the sheath. In either of the two cases, light may to a
greater or lesser degree interact with the subject
(external medium)and this phenomena is termed evanescent-
field coupling. A sensitive fiber consists therefore of a
segment of optical fiber which is placed in contact with
30   the surface of a biological medium, where the evanescent-

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field may be accessed externally and the propagating light interacts with this biological medium. The presence, growth and/or reproduction of microorganisms in the culture medium dynamically alter its optical constants.

5 This means that from the moment that the microorganisms are inoculated in culture medium it starts, in a general manner, to have its coefficient of attenuation and index of refraction varying with time and the surface or volume of the biological mass. The evanescent-field extends in

10 significant amplitude which characterizes a volume of light-microorganism interaction.

In the light-microorganism interaction, the optical signal through its evanescent-field shall, in a general manner, experience a temporal variation of the average

15 index of refraction (component DC) and the attenuation coefficient due to the intrinsic absorption of the medium and spreading (Mie) because of spatial fluctuations (component AC) of the index of refraction generated by the presence of each microorganism.

20 As such, when the microorganism starts its growth along the length of the optical fiber two effects may occur: (i) during the lag phase, due to the metabolism of the microorganism (for instance, bacteria) enzymes are released that cause an alteration of the index of

25 refraction and/or (ii) due to an increase in the number of microorganisms in contact with the optical fiber, during the log phase, the medium turns opaque with time. In this manner, the intrinsic absorption also suffers alterations. Therefore, the reduction of the optical power is

intimately related with the number of microorganisms present in the volume occupied by the evanescent-field around the fiber.

Therefore, the light propagated through the sensitive fiber simultaneously interacts with the microorganisms present and/or in evolution that are within the volume of interaction by coupling of the evanescent-field. In this manner, the actual mechanism of surveying occurs, where the optical signal is modulated in function of the temporal variations of the optical constants. Therefore, the presence and/or evolution of the microorganisms affects some characteristics of the light guided through the sensitive optical fiber. The characteristics of light that may be affected individually or in a combined manner are: amplitude (intensity), complete phase, wavelength or polarization. As can be seen in FIGURE 1, the sensitive fiber is inserted continuously (in-line) in an adequately constructed fiber optic circuit where the optical signal bearing information from the subject can be demodulated during the course of the biological culture. Some possible optical circuits are shown in FIGURE 1 and can demodulate signals that have been modulated at the propagation by the sensitive optical fiber by: average optical power (amplitude or intensity), complete phase (advance or delay of the phase), reflected and/or transmitted spectrum (wave-length), polarization and shape/temporal or spectral parameterisation of optical pulses. In this manner, it is possible to indirectly detect the presence of and/or temporally monitor the microorganisms, eventually inferring additional information such as, for instance,

the initial concentration of microorganisms, in the case of the sensor being calibrated beforehand.

The present invention consists, preferably, of an available material medium where possibly microorganisms may exist and/or be cultivated selectively. This material medium may constituted of a biological gel within a Petri dish in a manner as to permit that microorganisms be cultivated on the surface of the aforementioned gel. Preferably, an agent may be added to the culture medium that permits the maintenance of the residual humidity favoring thus, a better interaction between the microorganisms and the sensitive area. More preferably, this agent is glycerol in an appropriate concentration.

This material medium may also be constituted of fluids of a corporal nature such as water, blood, urine, etc. In this last case, microorganisms may be present and reproduce throughout the volume of the fluid. The segment of sensitive optical fiber should be conveniently placed on the surface or inserted in the volume of the biological culture medium. Beforehand, however, this fiber shall have an area of its primary covering removed and will undergo a treatment employing an appropriate technique based on chemical and physical properties. This treatment aims to expose the evanescent-field of the optical fiber through etching, for instance, using, preferably, strong acids, such as hydrofluoric, hydrochloric, sulfuric acids, for instance. It is necessary that the treatment lasts for the time necessary to etch the sheath of the optic fiber to an approximate thickness of 0.5 to 1 μm from the core. In

this way, it is possible to expose approximately 80% of the evanescent-field. The etching is interrupted by the immersion of the fiber in deionised water and, afterwards, in a pH stable solution for an adequate time to remove any

5   residue of water. Thus, it is possible to couple the evanescent-field of the means of guided propagation to the material medium containing the subject (microorganisms). The sensitive fiber optic, in turn, is inserted continuously in a larger fiber optic circuit, having a

10   particular assembly with the aim of demodulating the optical signal modulated by the subject. These characteristics confer to the invention operational advantages of biological selectivity, sensitivity, resolution, response velocity, stability, possibility of

15   miniaturization, ease of operation and easy incorporation into larger and more complex optical circuits that are destined to compose a survey network (overall survey) in a manner that microorganisms may be detected/monitored in various places simultaneously. The sensor technology

20   described here should be employed preferably for biological detection/monitoring in a qualitative and/or quantitative manner having a specific microorganism as subject.

The present invention may be employed, for instance,

25   in the detection/monitoring of aerobiological pathogens encountered in hospital, outpatients and clinic environments, in the bacteriological verification of foodstuffs in general, and in samples intended for verification of water quality.

The preferred arrangement for the invention described herein, acting as a sensor for microorganisms, may be constituted in various possible assembly as illustrated in FIGURE 1. The preferred arrangement does not exclude the possibility that the sensitive fiber optic apart from the core and sheath, may have incorporated to it one or more concentric layers of dielectric, metallic, semiconductor or superconductor materials with the end of altering the transversal spatial distribution of the evanescent-field and therefore optimizing the coupling with the biological medium and thus the performance of the sensor. More preferably, the interaction bacteria-fiber may be favored coating the fiber with a polymer film such as, for instance, polyvinyl chloride, polyurethanes, polyureas, and polyesters. In addition, the sensitive optical fiber may contain the engraving of a Bragg grating that works like a spectral filter. The Bragg gratings engraved in optical fibers are well described in Kashyap (Raman Kashyap. "Fiber Bragg Gratings". Academic Press, 1999) and are constituted by an axial modulation in the index of refraction of the core in lengths of, typically, millimeters or centimeters. Thus, part of the spectrum entering the Bragg grating shall be reflected by the fiber whilst the remaining spectrum shall be transmitted. Modulation of the wave length will be generated when changes in the index of refraction experienced by the propagating light in the sensitive fiber optic occurs. One means of obtaining this is well described by Ribeiro *et al* (R.M. Ribeiro *et al*. "All-optical control of Bragg grating in semiconductor coated D-shaped fiber". Optics Letters. 24.7. pp.111-113, 1999) where the light interacts

simultaneously with the Bragg grating and some material medium whose index of refraction suffers changes. The sensitive optical fiber may also be constituted as a fiber with a high birefringency (HiBi) such as, for instance, a

5 fiber maintaining polarization. In this manner, the propagating light experiences two different indexes of refraction in directions orthogonal to each other. In the case of the index of refraction of the subject as experienced by the evanescent-field suffers changes, light

10 polarization shall be modulated. The sensitive optical fiber, as well as being of the polarization maintaining type, may also contain an engraved Bragg grating. In this case, it will be possible to obtain the modulation in the wave length and the polarization simultaneously. The

15 preferred arrangement also does not exclude the possibility that the sensitive optical fiber may be inserted in some other type of assembly (fiber optic circuit for demodulation) not described by this patent. The optical fiber(s) used whether for the construction of

20 the optical circuit for demodulation or for the making of the sensitive fiber may be manufactured using as raw material silica (SiO_2) and other glass in general, plastics of various types (polymers) or any other material medium with a sufficient optical transparency.

25 FIGURE 1 shows the diagram of the preferred assembly of the present invention, in other words, the implementation of survey technology for microorganisms by fiber optics. An optical source (1) that may be constituted by an electrical luminescent diode (LED) or a

30 semiconductor laser (LD), possesses the function of

generating light in a continuous mode, modulating or
pulsing, that shall be used in the circuit of the
microorganism survey. The light produced by the optical
source is applied to the circuit through a fiber optic
coupling of the 2x1 type (2), also called a WDM coupling.
The other source of entry (3) of the 2x1 coupling is
constituted, in reality, by an exit, so that the light
processed by the survey device may be detected by the
photodetector (4). This photodetector is preferably
constituted by a semiconductor of the photodiode or
phototransistor type, which functions in the electrical
domain whereby light is transformed into a photocurrent.
The coupling (2) is linked by fusion or mechanical
connection to another 2x2 coupling to the optical fiber
(5), also termed bi-directional coupler. The other source
of entry (6) of the 2x2 coupling is constituted, in
reality, by an exit, so that the light processed by the
survey device may be detected by the photodetector (7).
This photodetector is preferably constituted by a
semiconductor of the photodiode or phototransistor type.
The exit of the coupling (5) is linked by fusion or
mechanical connection to the two extensions of optical
fiber, one is termed sensitive extension (8) and the other
is termed reference extension (9). Part of the optical
fiber of the sensitive extension (8) may be coiled around
a certain number of rings of a certain diameter, so as to
constitute a polarization controller (10) of the
propagating light. The sensitive optical fiber (11) is
linked by fusion or mechanical connection in a manner as
to provide continuity to the sensitive extension (8) of
the optical circuit for survey. This fiber permits that

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the evanescent-field be accessed when put in direct
physical contact with some biological medium (12). The
sensitive optical fiber may be connected at extremities
(13) and (14) to the rest of the optical circuit, termed

5   demodulation circuit. The exit (15) of the sensitive
extension (8) allows the light processed by the device to
be detected by the photodetector (11). This photodetector
will be constituted, preferably, of a semiconductor of the
photodiode or phototransistor type. The other exit of the

10   coupling (5) shall be linked by physical or mechanical
connection to the reference extension (9), where part of
the optical fiber that constitutes it is coiled around
another polarization controller (17). The exit (18) of the
reference extension (9) allows the reference light of the

15   device to be detected by the photodetector (19). This
photodetector will be constituted, preferably, of a
semiconductor of the photodiode or phototransistor type.
The light processed by the survey device emerges from the
exit (15) and is detected by the photodetector (16). This

20   photodetector will be constituted, preferably, of a
semiconductor of the photodiode or phototransmitor type.
The reference light emerges from the exit (18) and is
detected by photodetector 19. In this manner, the device
functions based on the modulation of the intensity

25   (amplitude) of light. The 2x2 fiber optic coupling (20)
possesses two entries (21) and (22). The exit (15) of the
sensitive extension (8) may be directly linked or
mechanically connected to the exit (18) of the reference
extension (9). In this case, the device formed shall be a

30   Sagnac fiber optic interferometer where the photodetector
(4) detects the reflected optical signal whilst the

photodetector (7) detects the optical signal transmitted by the device. If the exits (15) and (18) were to be reflective in a manner that light were at both extremities, the device formed will be a Michelson fiber optic interferometer where the photodetectors (4) and (7) will detect the processed optical signal. The links (13) and (14) can merely be done through the use of mechanical connections. In this case, the cleaved extremities of the sensitive optical fiber (11) may be semi-reflective, so that a fraction of the light may undergo multiple reflections in the sensitive optical fiber (11). In this manner, the device form shall be a Fabry-Perot fiber optic interferometer, where the signals shall be detected by photodetector (16) e (19). The exits (15) and (18) may be linked by fusion or mechanical connection to the entries (21) and (22), respectively, of the coupling (20). In this case, the device formed shall be a Mach-Zehnder fiber optic interferometer, where the signals shall be detected by photodetectors (23) and (24) after emerging from extremities (25) and (26) of the optical fiber.

The present invention is described in detail by the examples presented below. It is necessary to mention that the invention is not limited to these examples, but also includes variations and modifications within the limits of which it works.

Example 1: Exposure of the evanescent-field

So as to allow the exposure of the evanescent-field, 20cm of a multimode optical fiber, of graded-index, with a diameter of 62.5/125µm is attacked by a hydrofluoric acid

solution at 38%, during 11 minutes. In this manner, the etching of the fiber optic sheath occurs to an approximate thickness of 0.5 to 1 µm from the core. After this period, the chemical reaction is interrupted by immersion in deionised water and, afterwards, in a phosphate buffered saline solution, pH 7.4, for 15 minutes. This fiber shall be, then, placed in the support containing the medium in which the microorganisms are growing.

Example 2: Obtaining an air sample

An air sample is collected through the technique known to experts in the field as impaction-on-a-gel employing Merck MAS-100 equipment. In this manner, a sample of the ambient air was aspirated, through perforated plates, with the aid of a vacuum pump (with a volumetric flow rate of 100 litres per minute) for 30 seconds. The resulting air stream that carries particles with diameters inferior to 10 µm, was directed to the agar surface of a Petri dish. Eighteen collections were undertaken, during six random days, three collections per day, during two weeks. During the three first days, a specific culture medium for *Staphylococcus aureus* was used, and during the three remaining days, a specific medium for *S. pneumoniae* was used.

Example 3: Growth of microorganisms in a selective culture medium

The culture medium used for *Staphylococcus aureus* resistant to methicillin (MRSA) obtained in Example 2 was the Baird-Parker Agar (Difco Laboratories-Difco 0768-17-3)

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at an incubation temperature of 35°C. For *S. pneumoniae*, the culture medium used was Trypticase soya agar (Difco Laboratories, Detroit, Michigan - Difco 0026-17-1) supplemented with sheep blood at 5% at an incubation temperature of 35°C. In both the culture media glycerol at 0,2% was added, so as to avoid drying out the culture during the tests. For the growth of *E. coli* 0157:H7, the selective culture medium employed was the MacConkey sorbitol agar base (Difco Laboratories-Difco 0079-17-7), at an incubation temperature of 35°C.

Many tests were performed so as to calibrate the biosensor for its selectivity, using different initial values of bacteria (N_0). *E. coli* 0157:H7 available commercially in lyophilized form was reconstituted with 1.0mml of phosphate buffer saline solution , pH 7.4, and inoculated in various Petri dishes containing MacConkey sorbitol agar base, supplemented with glycerol at 0.2%. The incubation was at 35°C for a period of 24 hours. After this period, the purity of the material was confirmed and the Petri dishes were stocked at 4°C, for later dilution. For obtaining the dilutions with N_0 = 10, 20, 30, 40, 50, 60, 70 and 80 microorganisms used 100 µl of phosphate buffer saline solution (pH 7.4) and for values of N_0 90, 100, 200, 400 and 800 microorganisms, the volume used was 500µl of phosphate buffer saline solution (pH 7.4). For each sample the cells were counted using a Coulter (Beckman) counter that permits an accuracy of ±1%. Finally, the sensitive probe of Example 1 was inserted in the Petri dishes containing the media described above, in a dilution with a known number of microorganisms and the

measurement of points was initiated with the use of
systems adequate for the measurement/acquisition of data
known to experts in the field.

Example 4: Correlation of the growth with the estimated
number of bacteria

FIGURES 2 and 3 present, respectively, the optical
signal of the sensor for *Staphylococcus aureus* resistant
to methicillin and *S. pneumoniae*. Lines 1, 2 and 3
represent the output signal for samples 1, 2 and 3,
respectively. Line 4 correlates the growth with the
estimated number of bacteria, in accordance with the
following formula:

$$N(t) = N_0 \, 2^{t/GT}$$

where t is the time in minutes, N is the total number of
bacteria in the time t, N_0 is the initial number of
bacteria (equal to 94 for *Staphylococcus aureus* resistant
to methicillin (MRSA) and 91 for *S. pneumoniae*) and GT is
the time of generation (equal to 30 minutes for MRSA and
48 minutes for *S. pneumoniae*).

Observing FIGURES 2 and 3, one notes the presence of
three distinct phases: the first is a plateau where no
response is detected (Lag phase). The result for
Staphylococcus aureus resistant to methicillin and *S.
pneumoniae* demonstrate a lag phase of, approximately, 6
and 13 hours respectively. This different is due to the
different times of generation for each microorganism, in
other words, 30 minutes for *Staphylococcus aureus*
resistant to methicillin and 48 minutes for *S. pneumoniae.*

The second region presents a negative inclination. This is the phase of exponential growth of the bacteria. The third phase is characterized by a saturation of the volume that occurs around the sensitive fiber. This is not yet the stationary phase of the culture, since it continues to grow for a total period of 48 hours.

Example 5: Characterization of the sensitivity of the biosensor.

Various measurements were performed with the aim of characterizing the sensitivity of the biosensor, each one during an interval of 24 hours (1440 minutes) employing 13 different values of colony forming units (CFU) or initial number of bacteria (N_0). These values vary from 10 to 800 for samples of *E. coli* 0157: H7. FIGURE 4 plots the data of the temporal optical response, $I_{(out)}(t)$ (in arbitrary units) of three of these measurements (N_0 = 10, 80 and 800). The results of the optical measurements are reproducible. The reproduction is also observed in all the measurements corresponding to the different values of N_0 (Lag phase, Log phase and stationary phase). In the first phase of bacterial growth, a level DC $I_{(out)}(t)$ = I_{Lag} is observed with approximately the same temporal variation. The level I_{Lag} is related to the response of the biosensor, in which *E. coli* 0157: H7 remains in its lag phase during the time of the Δt_{Lag} decrease.

FIGURE 5 shows a plot, for all the measurements of the Δt_{Lag} data versus N_0. The linear ratio with practically no angular coefficient was field out with an average of 270±4 minutes or, approximately 4.5 hours,

represents a reproducibility of about 1.5%. Δt_{Lag} is attributed to the temporal variation that *E. coli* 0157: H7 consumes in its Lag phase independent of its initial number, N_0.

The optical attenuation ΔI_{out} (in dB) for each N_0 signifies the difference between the variation of time Δt_{Log} (in hours) of the Log phase, for each N_0. The derivative of the time β_{Log} of $I_{out}(t)$, in the Log phase, varies with N_0 and can be calculated by the following formula:

$$\beta_{Log}(N_0) \equiv \Delta I_{out}/\Delta t_{Log}.$$

FIGURE 6 demonstrates the sensitivity of the biosensor β_{Log} (dB/hour), during the Log phase, when the initial number of bacteria varies from $N_0=10$ to $N_0=400$. The linear correlation coefficient of 0.985 provides a straight line for the calibration curve: $\beta_{Log}(N_0) = (\Delta\beta_{Log}/\Delta N_0)N_0$. The calculated angular coefficient is $\Delta\beta_{Log}/\Delta N_0 = (0,016 \pm 0,001)$ (dB/hour)/bacteria. Thus, the response speed of the biosensor during the Log phase increases in 0.016dB/hour, for each *E. coli* 0157:H7 inoculated in the Petri dish. For $N_0=800$, the angular coefficient $\beta_{Log}(800) \approx$ dB/h. Therefore, it is possible to infer from the output signal the initial number of bacteria measuring the angular coefficient of the Log phase. The number of colony forming units is directly related to the degree of contamination of the sample. FIGURES 7 and 8 show photographs obtained by electronic microscopy of *E. coli* 0157:H7 (Log phase) in physical

contact with an optical fiber and with a phase contrast optical microscopy of *E. coli* 0157:H7, respectively.